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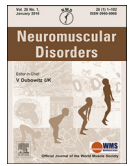
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# Myotonia congenita in a Labrador Retriever with truncated *CLCN1*<sup>☆</sup>

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## Abstract

An eight week old Labrador Retriever puppy presented with stiff-legged robotic gait. Abnormal gait was most evident after rest and improved with prolonged activity. On occasions, initiation of sudden movements would result in collapse with rigidity of the trunk and stiff extended limbs for several seconds. Other clinical signs were excitement-induced upper airway stridor and oropharyngeal dysphagia. Myotonia congenita was diagnosed based on clinical signs, abundant myotonic discharges on electromyography and exclusion of structural myopathies on histology. Whole exome sequencing revealed a case-specific homozygous variant in *CLCN1*, c.2275A>T resulting in a premature stop codon, p.R759X. The *CLCN1* variant was absent from the genomes of 127 Labrador Retriever controls and 474 control dogs from other breeds. This study expands the spectrum of identified canine *CLCN1* mutations and the list of affected breeds in myotonia congenita and highlights the potential value of dogs as translational large animal models of human genetic diseases.

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**Keywords:** *CLCN1*; Labrador Retriever; Myotonia congenita.

## 1. Introduction

Non-dystrophic myotonias are a group of skeletal channelopathies including myotonia congenita and the sodium channel myotonias (paramyotonia congenita, potassium-aggravated myotonia, hyperkalemic periodic paralysis among others) caused by mutations affecting skeletal muscle ion channels (*CLCN1*, chloride voltage-gated channel 1; *SCNA4*,

sodium voltage-gated channel alpha subunit 4) [1]. The non-dystrophic myotonias are distinguished from the dystrophic myotonias, which present as multisystem disease with variable extramuscular manifestations, progressive muscle weakness, muscle wasting and myotonia [2,3].

Myotonia congenita in humans is an inherited skeletal muscle ion channel disorder, due to a mutation in the sarcolemmal voltage-gated chloride channel gene (*CLCN1*) on chromosome 7q35 [4]. More than 100 missense and non-sense mutations, insertions, deletions and splice site mutations in *CLCN1* have been identified in humans [1]. Myotonia congenita is characterized by delayed relaxation of muscles after voluntary contraction and reflects a state of muscle fiber hyperexcitability. In humans, the inheritance is autosomal recessive (Becker type, severe) [5] or autosomal dominant (Thomsen type, mild) [6]. Both forms are characterized by muscle stiffness, typically provoked by gait initiation or sudden movements after rest. Myotonia severity improves after continued activity, the

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so called “warm-up phenomenon”. Additionally, it presents as percussion myotonia with prolonged muscle contraction after mechanical compression (reflex hammer). The recessive form shows more severe clinical signs, patients experience transient weakness on initiation of movement and display a more pronounced muscle hypertrophy [5,6]. Electromyography shows typical myotonic discharges with waxing and waning frequency and amplitude and the characteristic “dive-bomber” sound on a loudspeaker [7].

Myotonia congenita has been extensively studied in goats [8,9] and thereafter in many other mammalian species including horses [10,11], calf [12], mice [13], dogs [14–16], cats [17,18], Murrah water buffalo [19] and sheep [20]. Like in humans, myotonia associated mutations were allocated to *CLCN1* in the goat [9], horse [11], mice [13], dog [15,16,21], cat [18], Murrah water buffalo [19] and sheep [20]. Amongst canine breeds, myotonia congenita has been described in Chow-Chows [14] and Miniature Schnauzers [15] and isolated cases have been identified in a Staffordshire terrier [22], Great Dane [23], Cocker Spaniel [24], Australian Cattle Dog [16] and Jack Russel Terrier [21]. All these breeds share a common phenotype with clinical myotonia featuring a warm-up phenomenon, electromyographic myotonia, absence of muscle changes apart from pronounced muscle hypertrophy. Genetic investigations have discovered variants in *CLCN1* in Miniature Schnauzers and Jack Russel Terriers (*CLCN1*: c.803C > T resulting in p.T268M) [15,21], the Australian Cattle Dogs and Border Collies (*CLCN1*: c.2665insA resulting in loss of the C-terminal 88 amino acid residues) [16,25] and cats (*CLCN1*: c.1930+1G > T resulting in an altered splice site) [18].

We present here a previously unreported myotonia congenita in a Labrador Retriever caused by a novel *CLCN1* mutation. This study increases the spectrum of genetic myopathies in Labrador Retrievers and enables genetic testing as a diagnostic and breeding tool.

## 2. Patients and methods

### 2.1. Clinical assessment

The signalment and history of the puppy were obtained and a clinical and neurological examination following standard procedures was performed. Blood was collected via venous puncture in EDTA, serum and heparinized tubes and submitted for further analysis. Urine was collected via cystocentesis. Routine laboratory evaluation included blood count (leukocytes, erythrocytes, thrombocytes, PCV, differential count), biochemistry (AP, ALT, creatine kinase, glucose, BUN, creatinine, sodium, potassium, chloride, calcium, phosphate, postprandial ammonia, bile acids, cholesterol, triglycerides), blood gas analysis (pH,  $\text{HCO}_3^-$ , ionized calcium, lactate) and urinary analysis (pH, specific gravity, sediment, protein:creatinine ratio) in an in-house laboratory. Serum was submitted for type 2M antibodies associated with immune-mediated masticatory muscle myositis (Neuromuscular Di-

agnostic Laboratory, San Diego, USA) and total thyroxine (IDEXX).

PCR based genetic analysis at a commercial laboratory (Laboklin, Germany) was performed for known disease variants in Labrador Retrievers: X-linked myotubular myopathy (*MTM1*, c.465C > A; exon 7) [26], the SINE exonic insertion mutation in PTPLA associated with centronuclear myopathy [27], and congenital myasthenic syndrome (*COLQ*, c.1010T > C, exon 14) [28].

### 2.2. Further investigations

All subsequent investigations were performed under general anesthesia following placement of an intravenous catheter into the cephalic vein, premedication with butorphanol (Dolorex, MSD Animal Health, 0.3 mg/kg) and diazepam (Ziapam, Ecuphar, 0.3 mg/kg). General anesthesia was induced with propofol (Narcofol, Cp-pharma, repeated 1 mg/kg boli) and maintained with isoflurane.

Electromyography (EMG) was performed with standard electrodiagnostic equipment (Viking Quest, Natus Europe, Planegg, Germany) and a concentric needle electrode (recording area 0.07 mm<sup>2</sup>). All the appendicular and axial skeletal muscles as well as muscles of the head and tongue were explored. Compound muscle action potential, motor nerve conduction velocity and F-waves were evaluated following stimulation of the tibial nerve at distal (hock) and proximal (trochanter) stimulation sites with monopolar needle electrodes. Compound muscle potential was recorded of the plantar interosseous mm. with surface electrodes placed in a standard tendon-belly recording [29]. Compound muscle action potential was evaluated following single and repetitive (3 Hz) tibial nerve stimulation.

Radiographs of the cervical spine were performed in a lateral and ventrodorsal position. Magnetic resonance imaging (MRI) of the brain and cervical spine was performed with a 1.5 T MR unit (MAGNETOM Symphony, Siemens, Erlangen, Germany) utilizing T2-weighted, pre- and post-contrast (gadodiamide, Omniscan<sup>TM</sup>, GE Healthcare, 0.3 ml/kg) T1-weighted and fat suppressed sequences.

### 2.3. Muscle and nerve biopsies

Skeletal muscle biopsies were taken from both thoracic and pelvic limbs (right biceps brachii, triceps brachii and gastrocnemius muscles). Moreover, a nerve biopsy was obtained from common peroneal nerve. The samples were shipped fresh to the laboratory for immediate processing. Muscle biopsies were snap-frozen in isopentane cooled in liquid nitrogen for cryohistology. Further samples were immersed in 10% neutral-buffered formalin for paraffin embedding and in 6.5% glutaraldehyde for semithin histology, on epoxy sections, and electron microscopy. Cryosections were stained with haematoxylin-eosin (HE), Engel's modified Gomori trichrome stain, oil red O, and periodic acid Schiff reaction. Moreover, fibre type differentiation was achieved through immunohistochemistry for myosin heavy chain. Fur-

ther slides underwent enzyme histochemistry for COX and NADH-TR activities. Paraffin sections were stained with HE and Goldner's trichrome stain.

Semithin sections were stained with toluidine blue-safranin O. Ultrathin sections of 50 nm were performed on selected areas of two muscles, contrasted with lead citrate and uranyl acetate and examined through electron microscopy (Zeiss EM10®).

Complementing respective genetic analyses, immunohistochemical staining was performed for dystrophin (mouse anti-dystrophin antibody, Novocastra Reagents) and spectrin (mouse anti-spectrin alpha chain antibody clone AA6, Merck Millipore) in both cryosections and paraffin sections.

Nerve samples were fixed in 2.5% glutaraldehyde and processed routinely to enable teased nerve studies and evaluation of semithin sections [30].

#### 2.4. Clinical course and treatment response

Follow-up data were obtained by regular contact to the dog's owner who provided videos of the gait and repeated examinations at LMU Munich. All the clinical examinations were performed with the owner's consent. The owner gave written permission for the scientific use and publication of data and multimedia files.

#### 2.5. DNA isolation and whole exome sequencing

##### 2.5.1. Study cohorts

EDTA-blood samples were collected from the case and privately owned dogs in Germany and Finland. The samples were stored at  $-20^{\circ}\text{C}$  until genomic DNA was extracted using the semi-automated Chemagen extraction robot (PerkinElmer Chemagen Technologie GmbH). DNA concentration was determined either with the NanoDrop ND-1000 UV/Vis Spectrophotometer or Qubit 3.0 Fluorometer (Thermo Fisher Scientific Inc). A sample collection was ethically approved by the Animal Ethics Committee of the State Provincial Office of Southern Finland, Hämeenlinna, Finland (ESAVI/7482/04.10.07/2015).

##### 2.5.2. Genetic analyses

The known variants for congenital neuromuscular diseases in Labrador Retrievers in the *PTPLA*, *MTM1* and *COLQ* genes were tested in a commercial laboratory (Laboklin, Germany). Libraries for whole exome sequencing were generated with SeqCap EZ developer design 140702\_canFam3\_exomeplus\_BB\_EZ\_HX1 (Roche) [31], according to the manufacturer's instructions. The sequencing was performed using Illumina's NextSeq500 with an average coverage of 68X. The alignment and variant calling has been described earlier [32]. Canine genome build CanFam3.1 was used as a reference sequence. Variant filtering was done with the assumption of a recessive mode of inheritance and the variants from the case dog were filtered against variants in 268 available unaffected control dogs (Supplementary Table 1). The presence of the candidate causative vari-

ant was further screened in the whole genome sequencing data available from 39 unaffected Labrador Retrievers and 229 other dogs from 63 breeds (Supplementary Table 1). Additional 88 unaffected Labrador Retrievers were genotyped by PCR and Sanger sequencing with the following forward 5'-CCAGTTCTTAGAGGCATGCC-3' and reverse 5'-GGGTGTCTATGGAGGTGGAG-3' primers. The genotype of the case dog was confirmed by Sanger sequencing.

### 3. Results

#### 3.1. Clinical presentation

An 8-week-old male Labrador Retriever puppy was presented with a history of intermittent stiff-legged robotic gait and straight-legged stance which was noted soon after the puppy became ambulatory. Additionally, excitement-induced upper airway stridor and difficulties swallowing liquids and food were reported. The dog was rescued from poor housing conditions and a detailed patient history was not available. Abnormal findings on physical examination included weak closure of the jaw, hypertrophy of the tongue (Fig. 1A) and continuous wheezing sounds, which worsened with excitement and manual compression of the larynx. The puppy displayed marked stiff-stilted gait in all four limbs with a decreased ability to flex the joints which was most evident after a short period of rest. When excited the puppy would start walking with a bunny hopping gait of the pelvic limbs for the first two to three steps. The gait improved and stiffness subsided with prolonged continued activity. This was considered a "warm-up phenomenon" typical for myotonia congenita (Videos S1 and S2). Percussion of the cranial tibial muscle with a reflex hammer elicited a sustained dimpling of the muscle and flexion of the hock for several seconds (Video S3). Mild discomfort was elicited at palpation of the dorsal neck, most pronounced at the level of the atlantooccipital joint. When fed dry dog food (kibbles), the puppy struggled to move the treat backwards with its tongue which appeared contracted for several seconds (Video S4). The neurologic examination was otherwise normal. Besides a mild increase in creatine kinase activity (697 U/l; reference range 54–348 U/l) the laboratory evaluation was unremarkable. Type 2M antibodies were absent and thyroxine hormone concentration was within the reference range of the laboratory. A few weeks later the owner reported that the puppy experienced collapsing episodes which were induced by violent playful activity (Video S5). Provided video sequences showed episodes with rigidity of the whole body and stiff extended limbs. After a fall on the ground the puppy remained in lateral recumbency with all four limbs extended, not able to move for several seconds. The dog recovered rapidly and returned to ambulation.

#### 3.2. Electromyography and magnetic resonance imaging

Electromyography identified prolonged insertional activity followed by abundant myotonic discharges in all mus-





Fig. 1. Labrador Retriever with myotonia congenita. (A) Hypertrophy of the tongue, (B) normal appearance of the head, (C,D) severe hypertrophy of the neck muscles, (E,F) moderate hypertrophy of the proximal fore and hind limb muscles.

cles with characteristic waxing and waning amplitudes in the anesthetized dog. The generated sound from these discharges on the EMG loudspeaker resembled a “dive bomber” sound. Myotonic discharges were best identified when displayed at higher sweep speeds (Video S6). Sciatic-tibial motor nerve conduction velocity (51 m/s) and repetitive tibial nerve stimulation at 3 Hz (no decrement or increment) were considered unremarkable for the age of the dog. Compound muscle action potential (recorded from the interosseous muscles with surface electrodes) was of normal amplitude and duration without any evidence of temporal dispersion.

Further investigations with MRI and radiographs failed to identify an underlying cause for the mild cervical pain besides mild atlantooccipital joint effusion and synovial contrast enhancement. All muscles appeared with normal signal intensity.

### 3.3. Muscle and nerve findings

Epimysium, perimysium and endomysial connective tissue, including blood vessels and intramuscular nerve twigs, were unremarkable. Myofibre density was within normal limits (Fig. 2A). Fibre diameters displayed variations featuring isolated or clustered small polygonal fibres (Fig. 2B–D, white asterisks) intermingling with mildly hypertrophic round non-lobulated myofibres (Fig. 2B–D, black asterisks). The myofibrillar pattern was preserved (Fig. 2B–D). Mitochondrial distribution and enzyme reactivities (Fig. 2B,C) were within a physiological range. The number of myonuclei was within normal limits but nuclear internalization was occasionally

seen (Fig. 2D). Some fibres showed a mild increase in interfibrillar fat droplets (Fig. 2D). Myofibre typing revealed 50% type 1 and 50% type 2 myofibres. Immunohistochemistry for dystrophin and spectrin showed continuous sarcolemmal staining pattern in both cryo- and paraffin sections. Transmission electron microscopy revealed a normal ultrastructure of myofibres, blood vessels and intramuscular nerve endings. No pathological changes were seen in the peripheral nerve specimen. Degree of myelination was in accordance with the age (Fig. 2E).

### 3.4. Clinical course and treatment response

Myotonia congenita was considered based on clinical signs, electromyographic myotonia and unremarkable muscle histology. Treatment with mexiletine hydrochloride at 4 mg/kg BID titrated up to 8 mg/kg BID (Novo-Mexiletine, Novopharm, Toronto, Canada) was initiated and has continued up to now. No side effects of the medication were noted and an electrocardiogram (after 2 months) was unremarkable. The owner reported the dog's improved ability to move the tongue and swallow food and less inspiratory noises were noticed. Initiation of movement appeared also improved, but muscle stiffness and abnormal gait after rest were still evident and collapsing episodes did not cease. Stiffness appeared worse on outdoor walks on cold days during the winter. Over the course of the following 12 months the puppy developed an impressive generalized muscle hypertrophy, most pronounced in the neck and proximal appendicular muscles of all limbs, but the appearance of the head remained normal (Fig. 1B–F).

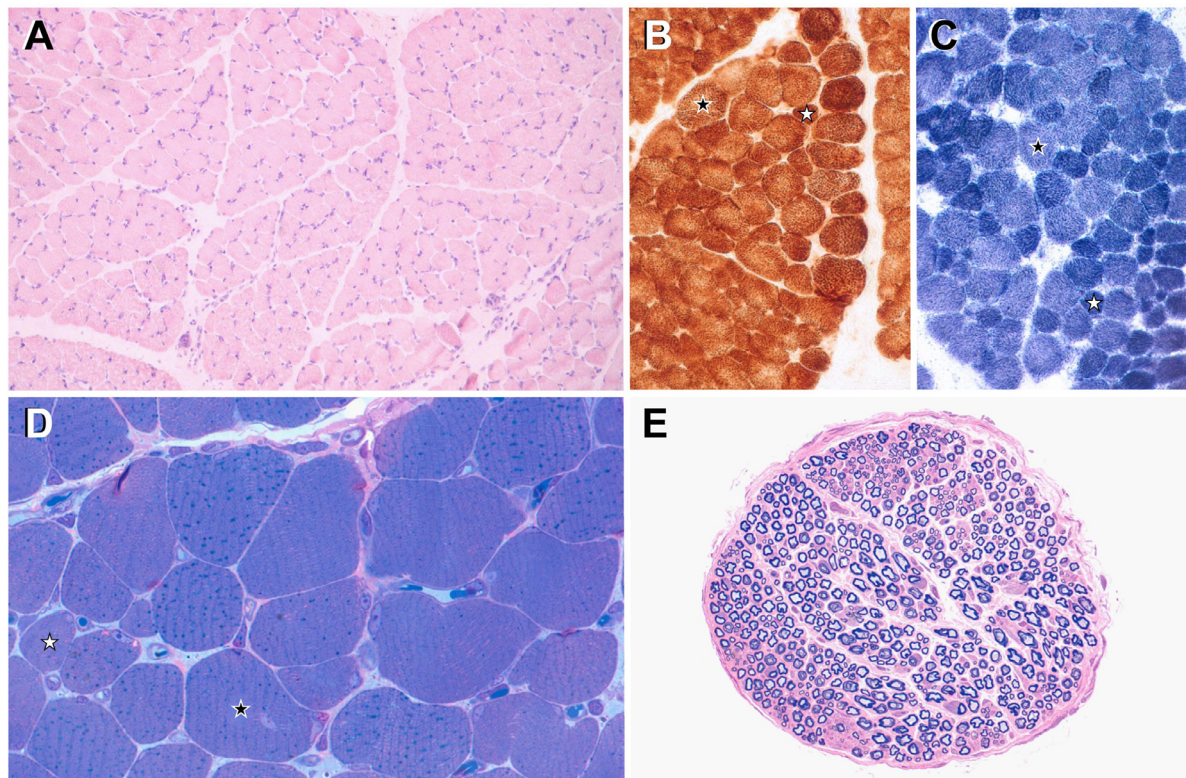


Fig. 2. Overview of muscle (A–D) and nerve (E) findings on cryosections (A–C) and semithin histology (D,E). Low power field examination of the muscle on HE as well as reactivities of cytochrome oxidase (B) and NADH-TR (C) show subsignificant changes. On close examination there are mild bimodal fibre size variations (B–D, asterisks). No changes are seen in nerve sections (E).

Stains: A: HE, B: COX, C: NADH-TR, D,E: azure II methylene blue-safranin O.

### 3.5. Genetic analyses

Since the known neuromuscular disease variants in the *MTM1* [26], *PTPLA* [27] and *COLQ* [28] genes were excluded in the affected dog, a whole exome sequencing was performed to identify the causative gene and variant. The variant data from the case dog were filtered against 268 unaffected dogs (Supplementary Table 1), resulting in the identification of six case-specific coding variants (Supplementary Table 2) of which the nonsense variant in *CLCN1* appeared most likely causative, since this gene has been previously associated with myotonia in dogs and humans. A homozygous nonsense variant in exon 19 (out of 23) of the *CLCN1* gene, c.2275A > T leads to a premature stop codon, p.R759X (Fig. 3). The *CLCN1* variant was absent from the genomes of additional unaffected 127 Labrador Retrievers and 474 control dogs. Exome sequencing data is available at Sequence Read Archive (SRA) under study accession SRP139271.

## 4. Discussion

This study describes a novel *CLCN1*-associated myotonia congenita in an 8-week-old Labrador Retriever. The family history of the affected Labrador Retriever puppy was unknown and samples from the parents and the siblings were not available for genetic analysis. Population screening failed

to identify any carriers. Thus, we cannot exclude a possibility of a de novo mutation in this puppy.

This study enhances the spectrum of congenital myopathies reported in Labrador Retrievers. Labrador Retrievers are affected by various types of rare genetic myopathies with overlapping clinical features, including X-linked myotubular myopathy (*MTM1*) [26], Duchenne muscular dystrophy (*DMD*) [33], centronuclear myopathy (*PTPLA*) [27], congenital myasthenic syndrome (*COLQ*) [28] and myotonia congenita (*CLCN1*) described here. The genetic basis remains to be identified for a mild muscular dystrophy [34] and sarcolemmal-specific collagen VI deficient myopathy in the Labrador Retriever [35], which has been attributed to *COL6A1* in Landseer dogs [36]. In Labrador Retrievers, electrical myotonia is also a feature of centronuclear myopathy [37]. Electromyographic myotonia was described in incidental cases of myotonic dystrophy in other dog breeds (Boxer, Rhodesian Ridgeback) [38,39] and with various toxins, metabolic and undefined myopathies [40]. The clinical signs in this Labrador Retriever with myotonia congenita are also reminiscent of two previously described movement disorders in Labrador Retrievers: familial reflex myoclonus (hyperekplexia, startle disease) and hypertonicity syndrome with extreme generalized muscle stiffness [41,42]. Electromyography failed to identify any myotonic discharges in the anesthetized dogs therefore myotonia congenita was excluded from both disorders. Despite



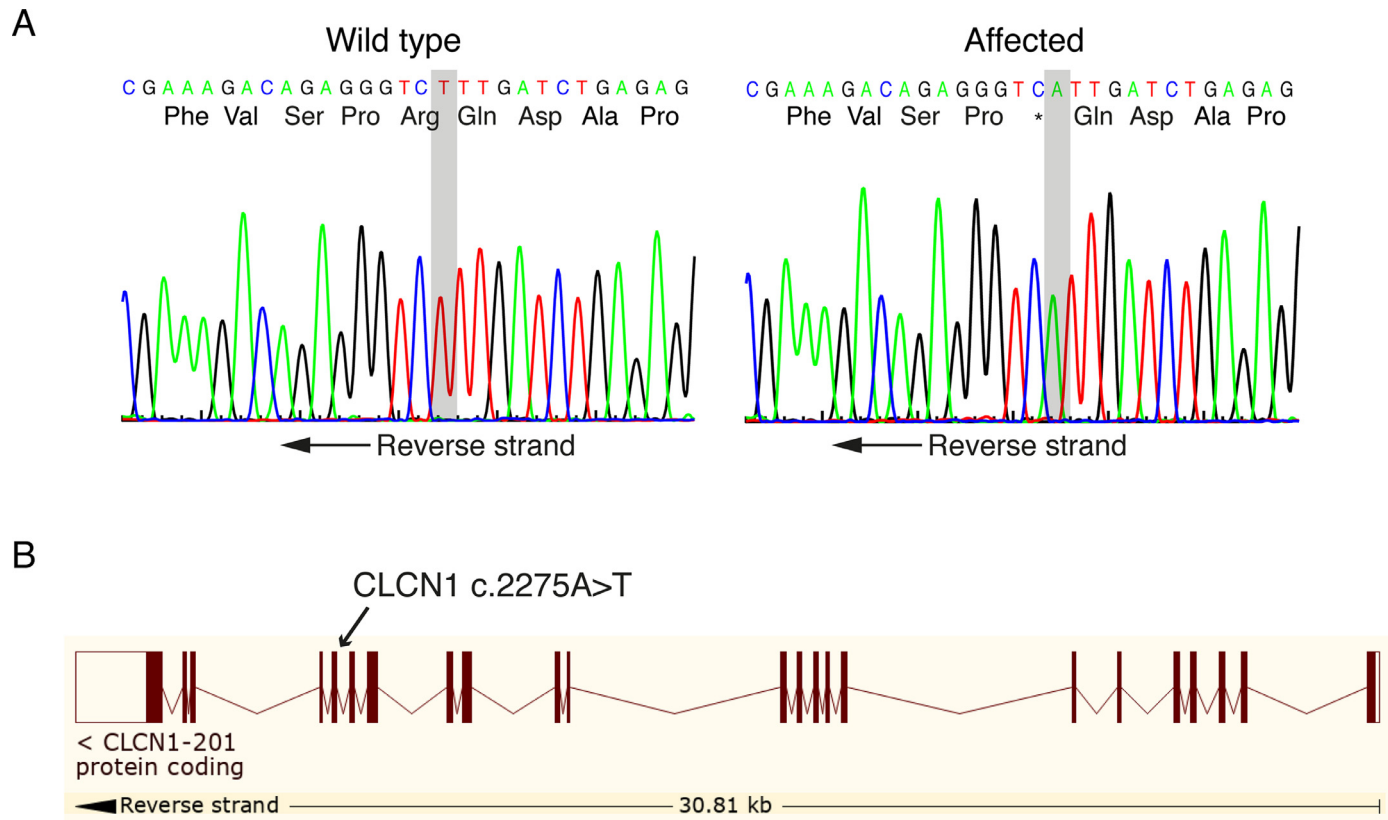


Fig. 3. Whole exome sequencing of a case dog revealed a homozygous nonsense variant (A) (c.2275A>T) in exon 19, resulting in a premature stop codon (p.R759X) and truncation of *CLCN1* (B).

the fact that the *CLCN1* variant found here was present only in one dog, the genetic test that can be developed will assist the differential diagnosis of clinically similar myopathies in the breed.

In the present case myotonia congenita was diagnosed based on characteristic clinical signs: muscle stiffness after rest, collapse provoked by sudden movements and abundant myotonic discharges with a typical waxing and waning pattern in the anesthetized dog. The observed clinical signs, stiff “robotic gait”, which improved with continued activity, transient immobility on initiation of sudden movements and prominent muscle hypertrophy are identical to other reports of myotonia congenita in dogs and humans [1,14–16,21]. Muscle and nerve biopsies and MRI primarily served to exclude myotonic dystrophies and other myopathies. Thus the diagnosis of myotonia congenita relied solely on demonstration of clinical and electromyographic myotonia and genetic findings. Brachygnathia and other craniofacial and dental abnormalities were described in other dogs with myotonia congenita, but were not seen in the Labrador Retriever [16,43]. Oropharyngeal dysphagia was the presenting complaint and was attributed to spasms of tongue and pharyngeal muscles and hypertrophy of the tongue. Oropharyngeal dysphagia is a frequent and often fatal complication of many congenital and acquired myopathies in humans and dogs [26,39,44–48]. Swallowing problems, stridor and decreased tongue mobility were described in the Miniature Schnauzer and cats

with myotonia congenita [40,18] and oropharyngeal dysphagia was the initial clinical sign in a Rhodesian Ridgeback with dystrophic myotonia [39]. This study provides further evidence that myotonia congenita should be considered as a possible cause for dysphagia and swallowing problems in puppies and that electromyography, muscle biopsies and genetic investigations are indicated. Genetic testing for known mutations and possibly also sequencing of the candidate *CLCN1* for new variants may be a convenient option for first line diagnostics for future suspected cases of myotonia congenita.

Specific treatment acting on the chloride channel is not available. Therefore, treatment of myotonia congenita aims to modulate the sodium channels to decrease the excitability of the muscle membrane. Mexiletine hydrochloride is the first-line treatment. Efficacy and side effects of other antiarrhythmic agents, antiepileptic drugs, antidepressants, calcium channel blockers, diuretics and amino acids have been explored [1,49]. Class 1 antiarrhythmic drugs such as mexiletine and procainamide have both been used successfully in the management of myotonia congenita in people and dogs [3,49,50]. Oral application of mexiletine hydrochloride was well tolerated by the Labrador Retriever. The owner reported the dog’s improved ability to move the tongue and swallow food with less inspiratory noises, but collapsing episodes and muscle stiffness were still observed. The veterinary literature describes treatment of myotonia congenita in a Miniature Schnauzer with mexiletine 8.3mg/kg every 8 h, resulting in

improvement of clinical signs [40]. A similar effect was seen with procainamide 40 mg/kg every 6 h, but higher dosages produced weakness and lethargy [51]. Extended-release procainamide 40–50 mg/kg every 8–12 h reduced clinical myotonia [40]. In Chow-Chow puppies quinidine and phenytoin alleviated myotonia, but procainamide showed the better effect [14,40]. Recently a randomized, double-blind, placebo-controlled study with lamotrigine (sodium channel blocker) showed effective reduction of myotonia in genetically confirmed myotonia congenita and paramyotonia congenita in humans [52]. Cardiotoxicity limits the use of lamotrigine in dogs [53].

Dogs represent an important translational animal model for preclinical studies evaluating new treatment modalities for congenital myopathies. Recent studies highlight the success of gene therapy in Golden Retriever muscular dystrophy and Labrador Retriever X-linked myotubular myopathy [54,55]. This study demonstrates the value of a joint clinical and molecular genetics approach with whole exome sequencing for the molecular diagnosis of genetic neuromuscular diseases in dogs.

## 5. Conclusion

Several genetic myopathies with overlapping clinical features have been reported in Labrador Retrievers. We describe a novel mutation in *CLCN1* causing myotonia congenita in the breed which can be included in future panel diagnostics for genetic myopathies in this dog breed. Genetic testing will offer a feasible approach for differential diagnosis in neuromuscular disorders particularly when myotonia congenita is suspected based on neurolocalization, phenomenology and EMG.

### Video S1 Gait of the Labrador Retriever puppy

The Labrador Retriever puppy displayed marked stiff-legged gait in all limbs which was most evident after a short period of rest. When excited the puppy would start walking with a bunny hopping gait of the pelvic limbs for the first two to three steps. The gait improved and stiffness subsided with prolonged continued activity consistent with a “warm-up phenomenon” typical for myotonia congenita.

### Video S2 Gait of the adult dog

The one-year-old Labrador Retriever shows stiff gait on the initiation of movements. The gait improves with continued activity (“warm-up phenomenon”).

### Video S3 Percussion myotonia

Percussion of the cranial tibial muscle with a reflex hammer elicited a dimpling of the muscle and sustained contraction of the muscle.

### Video S4 Oropharyngeal dysphagia

When feeding dry dog food (kibbles), the puppy struggles to move the food backwards with the tongue. The second part of the video shows problems with the uptake of water and repeated swallowing efforts.

### Video S5 Collapsing episodes

The three-month-old puppy experienced collapsing episodes, which were induced by violent playful activ-

ity. Video sequences show episodes with rigidity of the whole body and stiff extended limbs. After a fall on the ground the puppy remained in lateral recumbency with all four limbs extended and was not able to move for a few seconds. The second part of the video shows the one-year-old Labrador Retriever collapsing for few seconds when initiating a sudden movement and a quick change of direction.

### Video S6 Myotonic discharges

Electromyography identified prolonged insertional activity followed by abundant myotonic discharges in all muscles with characteristic waxing and waning amplitudes. The generated sound resembled a “dive bomber” appearance. Myotonic discharges were best identified when displayed at higher sweep speeds.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.nmd.2018.05.002](https://doi.org/10.1016/j.nmd.2018.05.002).

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